U.S. Department of Energy's
Radiation Health Effects Studies in the Russian Federation

Proposal Number:	98-0009
Principal Investigator:	Belinsky, Steven A.
Institute:	Lovelace Biomedical & Environmental Research Institute
Title of Proposal:	Molecular Epidemiology of Lung Cancer in Workers from the MAYAK Nuclear Enterprise.

Abstract

The molecular mechanisms and gene environment interactions leading to radiogenic lung cancer have not been well characterized. The proposed collaboration between scientists at the Branch 1 Center of Biophysics (FIB-1), Ozyorsk, Russia, and the Lovelace Respiratory Research Institute, Albuquerque, NM, will facilitate a detailed molecular epidemiology study of workers from the MAYAK nuclear enterprise. These workers were gamma irradiated and exposed to airborne ^{239}plutonium. Lung cancers in exposed workers were frequently found in the lower and middle lung lobes and at the periphery, thus reflecting the deposition pattern for inhaled plutonium. Epidemiology studies indicate a significantly higher frequency for adenocarcinoma in workers than in a control population and a strong correlation between this tumor type and plutonium exposure. Two hypotheses will be evaluated through the proposed studies: (1) radiation exposure targets specific genes for inactivation and these genes can serve as biomarkers for lung cancer risk in population-based studies of exposed workers and (2) inherent deficiency in the repair of DNA double strand breaks and/or the detoxification of eletrophilic intermediates, constitute risk factors for lung cancer associated with radiation-induced exposure. The first hypothesis will be tested using adenocarcinomas collected both retrospectively and prospectively from exposed workers and an unexposed population. Because activation of protooncogenes and inactivation of tumor suppressor genes by point mutations are not found in most adenocarcinomas, the role of an epigenetic mechanism, specifically the inactivation of tumor suppressor genes by CpG island promoter methylation, will be examined. In support of hypothesis one, recent findings from our laboratory have shown that inactivation of tumor suppressor genes by CpG island methylation can be modulated differentially depending upon carcinogen exposure. Specifically, inactivation of the estrogen receptor (ER) by CpG island methylation was greater in never-smokers than in smokers and greater in rodent tumors induced by radiation than by the tobacco-specific nitrosamine NNK. Moreover, methylation of the ER and p16 genes has been detected in exfoliated cells within sputum from cancer-free uranium miners, which suggests that these two genes could serve as biomarkers for identifying high-risk persons in this cohort. The second hypothesis will be tested in an prospective study of exposed lunch cancer patients and cancer-free former workers. The activity of DNA protein kinase (DNA-PK), which is involved in the detection and repair of DNA damage, will be measured in peripheral blood lymphocytes. The genomic status of the glutathione-S-transferase μ gene, one of a family of enzymes involved in detoxificaiton of eletrophilic intermediates, will be determined in DNA from peripheral blood lymphocytes. In support of hypothesis two, recent studies in our laboratory have demonstrated interindividual variation in DNA-PK activity and a potential association to lung cancer is now being examined. During Phase I of the proposed studies, scientists from Lovelace will interact with FIB-1 scientists to exchange ideas, determine sample availability and size of populations for the proposed studies, conduct onsite training, and work with actual tumor specimens to provide preliminary data. The work described in this proposal, if continued in Phase II, will begin to provide fundamental knowledge of the mechanisms leading to radiation-induced lung cancer associated with exposure to airborne plutonium. Substantiating a role for CpG island methylation in the development of radiation-induced tumors in the MAYAK cohort could have important implications for both early detection and intervention. First, we can examine exfoliated cells within sputum from exposed workers for methylated alleles of the p16 and ER gene. Finding these alterations could be a sign of occult disease or extensive premalignant lung damage. This finding could facilitate more aggressive monitoring or clinical examination to detect the lung cancer when it is still in an early curative stage. Second, the reversal of abnormal methylation patterns in tumor cells is a feasible objective for chemotherapy and prevention. Studies directed toward identifying whether DNA-PK activity or GST μ status predicts risk for radiation-induced cancer could also prove invaluable for defining risk for both the MAYAK workers and individuals living in the villages along the Techa River (received discharges from the MAYAK facility) who were exposed to Sr 89, 90, and Cs 137 through ingestion of river water and milk. Together, these studies represent an opportunity to begin to unravel the molecular epidemiology underlying radiation-induced adenocarcinoma of the lung.

PROTOCOL

OF THE RESULTS OF THE VISIT OF STEVEN BELINSKY, Ph.D., AND RICHARD CROWELL, M.D., TO THE FIRST INSTITUTE OF BIOPHYSICS AUGUST 31, 1998 THROUGH SEPTEMBER 6, 1998

INTRODUCTION

In the frame of the agreement Between the Government of the Russian Federation and the Government of the United States of America on Cooperation in Research on Radiation Effects for the Purpose of Minimizing the Consequences of Radioactive Contamination on Health and the Environment the visit of the American scientists to the First Institute of Biophysics (FIB-1) took place.

TITLE of the Project: "Molecular Epidemiology of Lung Cancer in Workers from the MAYAK Nuclear Enterprise."

The Russian team consisted of Vitaliy I. Telnov, M.D., Ph.D.; Galina G. Rysinova, Ph.D.; Nadezhda D. Okladnikova, M.D., Ph.D., Sc.D.; and Galina V. Adamova, M.D.

The American team consisted of Steven A. Belinsky, Ph.D. and Richard E. Crowell, M.D.

PURPOSE

The purpose of the visit was to establish a working relationship between the Russian and American teams and to develop a plan and strategy to carry out the Phase I studies detailed in the above-titled proposal. As a result of the visit, the Russian and American teams have identified common areas for which we will establish a new working collaboration. The visit has also helped the American team to understand the facilities and capabilities that exist at FIB-1, and has helped both teams to understand the type of prospective and retrospective collaborative studies that may be feasible to conduct.

RESULTS

As a result of the meeting, we have agreed on several objectives:

1. The American team will send protocols, reagents and accessories for DNA isolation from blood samples and sputum samples and for sputum induction to the Russian team as soon as possible on their return to the US after this meeting.

2. The Russian team will then use these DNA isolation protocols to modify the current protocols in place in their labs to isolate DNA from blood obtained from 5 control persons (without radiation exposure). These samples will be brought to the U.S. when the Russian team visits the labs of the American team for quantitative and qualitative evaluation of their techniques using American facilities.

3. The Russian team will identify and recover 5-10 paraffin blocks from lung cancer tissues which will be brought to the U.S. when the Russian team visits the labs of the American team.

4. The Russian team will visit the laboratories of the American team in January or February of 1999. During this visit, the American team will review DNA isolation techniques with

the Russian team including evaluation of DNA isolated in the Russian team labs, will review sputum induction techniques, and learn techniques for DNA isolation from cells in sputum, and evaluate genetic analysis laboratory procedures used by the American team.

5. On return to Russia, the Russian team will obtain blood and sputum from approximately 10 persons with high-dose plutonium burden. DNA will be isolated from the sputum and blood samples from each of these persons by the Russian team in their laboratories.

6. In addition, the Russian team will obtain pertinent clinical, occupational, and smoking information on these subjects important for epidemiological analysis.

7. At a time to be determined, these DNA samples from sputum and blood will be either sent back to the American labs or brought by members of the Russian team on a return visit to the US in the Spring, 1999, depending on time and budget restraints. These samples will then be analyzed for p16 and other gene abnormalities.

The success of this Phase I collaboration is dependent on the ability to acquire the necessary reagents for DNA isolation by the Russian team.

Also, the ability of the Russian team to work together with Drs. Romanov and Myksinova to identify the paraffin blocks from lung cancer tissues will be critical to the development of our group's future plans. This will also be essential for another project headed by Dr. Bigbee. This needs to be accomplished by September 1, 1999.

FUTURE PLANS

The future plans for this international collaboration are:

1. to evaluate the results of the Phase I studies
2. to develop the Phase II proposal based on the information gained from Phase I.

SIGNATURES

For the Americans

Steven A. Belinsky, Ph.D.

September 4, 1998

For the Russians

Vitaliy I. Telnov, M.D., Ph.D.

September 4, 1998